UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: August 7, 2015

Subject: Efficacy Review for Brace
EPA Reg. No. 777-99 (DP #427181)

From: Alison Clune
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

Thru: Mark Perry, Team Leader
Product Science Branch
Antimicrobials Division (7510P)

To: Jacqueline Hardy/Lorena Rivas, Team 34
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: Reckitt Benckiser, Inc.
Morris Corporate Center IV
399 Interpace Parkway
Parsippany, NJ 07054-0225

Formulation from the Label:

Active Ingredient(s)	% by wt.
Alkyl (50% C14, 40% C12, 10% C16) dimethyl benzyl ammonium saccharinate	0.10%
Ethanol	58.00%
Other Ingredients	49.90%
Total	100.00%

I BACKGROUND

The product, Brace (EPA Reg. No. 777-99), is an EPA-approved hard surface disinfectant (bactericidal, virucidal, fungicidal, tuberculocidal), non-food contact hard and soft surface sanitizer, and hard surface mildew fungistat and deodorizer for use in residential, commercial, and institutional environments. The applicant is submitting efficacy data from studies conducted at ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121.

This data package contained a letter dated April 17, 2015 from the applicant to EPA, a copy of the data matrix, 3 efficacy studies (MRID 49599201-49599203), and the proposed label. Statements of No Data Confidentiality Claims, Good Laboratory Practice, and Quality Assurance Unit Summaries were included with each study. The Confidential Statement of Formula (EPA Form 8570-4) dated conditionally acceptable 12/16/05 was used to find the lower certified limits of the active ingredients.

II USE DIRECTIONS

The product label indicates that it is designed for disinfecting hard, non-porous surfaces against the organisms tested in the submitted studies. Such hard, non-porous surfaces on the label include brass, chrome, copper, crystal, enamel, glass, glazed tile, laminate, linoleum, marble, marlite, metal, parquet, plastic, sealed granite, stainless steel, tin, vinyl, as well as floors, walls, fixtures, furniture, toys, and tools made of these hard non-porous materials. Use sites in which these hard, non-porous surfaces may be treated include homes, vehicles, garages, kitchens, bathrooms, campers, public places, supermarkets, and home workshops. Professional use sites on the label include hospitals and healthcare facilities, cafeterias, restaurants, schools, day care centers, laboratories, and commercial and office buildings.

Directions on the proposed label provide the following information regarding preparation and use of the product as a disinfectant:

"Pre-clean surfaces prior to use. Hold can (container) upright 6" to 8" from surface. Spray 3 to 4 seconds until covered with mist. ((Gross) (Heavy) soil must be removed prior to application.)…
For Wide Spray Can: (Pre-clean surfaces prior to use.) Hold can (container) upright 6" to 8" from surface. Spray (for) (X) (seconds) until (covered with) (thoroughly) (wet) (mist). ((Gross) (Heavy) soil must be removed prior to application.)…

To Disinfect: Surfaces must remain wet for 3 minutes then allow to air dry.

Rinse toys and food contact surfaces with potable water after use.

For surfaces that come in contact with food: Use only on hard, non-porous surfaces and rinse thoroughly with water.

To Disinfect Toys: Use only on hard, non-porous surfaces and rinse thoroughly with water after use. Surfaces must remain wet for 3 minutes then allow to air dry.)…
Rinse child/baby plastic toys, child/baby hard non-porous surfaces and all food contact surfaces with potable water or a damp cloth after use."

III AGENCY STANDARDS FOR PROPOSED CLAIMS

<u>Disinfectants for Use on Hard Surfaces, Additional Microorganisms:</u>

Efficacy of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method (for liquid products) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray products, or with modification for towelette products). Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots, both tested at or below the nominal concentration(s) of the active ingredient(s). Testing must be conducted against a minimum mean log density of 4 CFU/carrier.

To support products labeled as “disinfectants” for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required with no contamination of any carrier.

Virucides:

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants, or with modification for towelette products) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant, both tested at or below the nominal concentration(s) of the active ingredient(s), must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Supplemental Claims:

An antimicrobial agent identified as a “one-step” disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5% serum.

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. **MRID 49599201 “Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces”, by Shanen Conway. Virus: Enterovirus Type 68. Study conducted at ATS Labs. Study Completion Date: 11/13/14. Laboratory Study Identification Number: A17237.**

This study was conducted against the Fermon strain of Enterovirus type 68 (ATCC VR-561). Two batches (2083-058 and 2083-064) of the product, Brace, were tested according to the ATS Labs protocol “Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces”, protocol number REK01090814.ENTV, which follows ASTM Standard Method E1053-11. The two batches of the test substance were received ready to use from the sponsor with the concentrations of the active ingredients at or below the lower certified limits. Stock virus culture was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. Cells were disrupted and cell debris was removed by centrifugation at approximately 2000 rpm for five minutes at approximately 4°C. The supernatant was removed, aliquoted, and stored at ≤-70°C until use. One aliquot was thawed on the day of use and adjusted to contain a 5% fetal bovine serum organic soil load. Virus films were prepared by spreading 200 µL virus culture uniformly over the bottom of three separate 100 x 15 mm sterile glass petri dishes and dried for 20 minutes at 15.5°C in a relative humidity of 55%. For each product batch, one dried virus film was sprayed for 4 seconds at a distance of 6-8 inches and held covered for 1 minute at 20.0°C. The dried virus control film was completely covered with a 2.00 mL aliquot of test medium instead of the test substance spray. Immediately prior to the end of the exposure time, the virus films were scraped to resuspend the virus in the test substance and the contents of each petri dish were filtered through individual Sephadex columns to detoxify the mixture. Ten-fold serial dilutions of the

filtrates were assayed in quadruplicate for infectivity and/or cytotoxicity to WI-38 human lung cells (ATCC CCL-75). Cell cultures were inoculated and allowed to adsorb for 60 minutes at 36-38°C in a humidified atmosphere of 5-7% CO_2. Then 1.0 mL test medium was added to each culture well before incubation for seven days at 36-38°C in a humidified atmosphere of 5-7% CO_2. Subcultures were examined for the presence or absence of cytopathic effect, cytotoxicity, and viability. Controls included dried virus, cytotoxicity, and neutralization (both product batches) controls. Viral and cytotoxicity titers were calculated by the Spearman-Karber method.

2. **MRID 49599202 "AOAC Germicidal Spray Method", by Jamie Herzan. Bacterium: *Escherichia coli*. Study conducted at ATS Labs. Study completion date: 1/29/15. Laboratory Study Identification Number: A17592.**

This study was conducted against *Escherichia coli* (ATCC 11229). Two batches (2083-058 and 2083-064) of the product, Brace, were tested according to the ATS Labs protocol "AOAC Germicidal Spray Method", protocol number SRC62111114.GS.1, which follows AOAC Official Method 961.02. The two batches of the test substance were received ready to use from the sponsor with the concentrations of the active ingredients at or below the lower certified limits. One loopful of stock slant culture was transferred to 10 mL growth medium, mixed, and incubated for 24 ± 2 hours at 35-37°C. A 10 µL aliquot was transferred to 10 mL culture medium and incubated for 48-54 hours at 35-37°C. The final culture was vortex mixed and allowed to stand for at least 10 minutes before the upper portion of the culture was removed and pooled in a sterile vessel. Two mL of the final suspension was diluted with 2.00 mL growth medium and adjusted to contain a 5% fetal bovine serum organic soil load. Glass slide carriers placed in individual petri dishes padded with filter paper were inoculated with 10.0 µL of culture spread uniformly over an approximately 1" x 1" area. Inoculated carriers were covered and dried for 30 minutes at 35-37°C in 53.1% relative humidity. Carriers were sprayed with the test substance for 2 seconds at a distance of 6-8 inches and left undisturbed in a horizontal position for 2 minutes ± 5 seconds at 21°C and 20% relative humidity. Then excess liquid was drained from the carrier and each carrier was transferred to 20 mL aliquots of neutralizing subculture medium and shaken. Neutralized subcultures were incubated for 48 ± 2 hours at 35-37°C before being examined for the presence or absence of visible growth. Controls included purity, sterility, viability, neutralization confirmation (both product batches), and carrier population controls.

3. **MRID 49599203 "AOAC Germicidal Spray Method", by Kristen Niehaus. Bacterium: *Cronobacter sakazakii*. Study conducted at ATS Labs. Study completion date: 1/22/15. Laboratory Study Identification Number: A17593.**

This study was conducted against *Cronobacter sakazakii* (ATCC 12868). Two batches (2028-029 and 2028-030) of the product, Brace, were tested according to the ATS Labs protocol "AOAC Germicidal Spray Method", protocol number SRC62111114.GS.2, which follows AOAC Official Method 961.02. The two batches of the test substance were received ready to use from the sponsor with the concentrations of the active ingredients at or below the lower certified limits. One loopful of stock slant culture was transferred to 10 mL growth medium, mixed, and incubated for 24 ± 2 hours at 35-37°C. Two daily transfers of 10 µL of culture to 10 mL culture medium were performed and the final culture was incubated for 48-54 hours at 25-30°C. The final culture was vortex mixed and allowed to stand for at least 10 minutes before the upper portion of the culture was removed and pooled in a sterile vessel. For testing on 12/8/14, the culture was centrifuge concentrated from 80.0 mL to 8.0 mL and adjusted to contain a 5% fetal bovine serum organic soil load. For testing on 1/6/15, the concentration of the test culture was not adjusted and no soil load was added. Glass slide carriers placed in individual petri dishes padded with filter paper were inoculated with 10.0 µL of culture spread uniformly over an approximately 1" x 1" area.

Inoculated carriers were covered and dried for 32 minutes at 35-37°C in 50.3% relative humidity on 12/8/14, or for 30 minutes at 35-37°C at 50.3% relative humidity on 1/6/15. Carriers were sprayed with the test substance for 2 seconds at a distance of 6-8 inches and left undisturbed in a horizontal position for 2 minutes ± 5 seconds at 20-21°C and 9-22% relative humidity. Then excess liquid was drained from the carrier and each carrier was transferred to 20 mL aliquots of neutralizing subculture medium and shaken. Neutralized subcultures were incubated for 48 ± 2 hours at 25-30°C before being examined for the presence or absence of visible growth. To confirm the presence of the test organism, representative test and positive control subcultures were subcultured to Tryptic Soy Agar + 5% Sheep's blood and incubated for 1 day at 25-30°C and assayed for characteristic growth of the test organism on 12/10/14. Controls included purity, sterility, viability, neutralization confirmation (both product batches), and carrier population controls.

Note: Testing resulted in a failure of Batch 2028-029 on 12/8/14. Testing was repeated at the sponsor's request without the 5% organic soil load on 1/6/15. All results are presented here.

V EFFICACY RESULTS

Disinfection – Virucidal Efficacy

MRID No.	Organism	Description	Results		Dried Virus Control $TCID_{50}$
			Batch 2083-058	Batch 2083-064	
1 minute contact time					
49599201	Enterovirus type 68, Fermon strain (ATCC VR-561)	10^{-1} serial dilution	Cytotoxicity	Cytotoxicity	$10^{4.75}$
		10^{-2} to 10^{-6} serial dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.1mL	$\leq 10^{1.50}$	$\leq 10^{1.50}$	
		TCD_{50}/0.1mL	$10^{1.50}$	$10^{1.50}$	
		Log Reduction	≥3.25	≥3.25	

Disinfection – Bactericidal Efficacy

MRID No. (Test Date)	Organism	No. Exhibiting Growth/Total No. Tested		Average log_{10} CFU/Carrier
		Batch 2028-029	Batch 2028-030	
2 minute contact time				
49599202	*Escherichia coli* (ATCC 11229)	0/10	0/10	4.00
49599203 (12/8/14)	*Cronobacter sakazakii* (ATCC 12868)	1/10	0/10	6.23
49599203 (1/6/15)	*Cronobacter sakazakii* (ATCC 12868)	0/10*	NA	5.49

* No organic soil load was added to the test organism suspension.

VI CONCLUSIONS

1. The submitted efficacy data **supports** the use of the product, Brace, as a disinfectant with virucidal activity on hard, non-porous surfaces in its ready to use form in the presence of a 5% organic soil load for a 1 minute contact time against the following:

   Enterovirus type 68 (ATCC VR-561) MRID 49599201

   According to the analysis of the active ingredient concentration for each product batch, the tested concentrations were at or below the lower certified limit of the active ingredient. Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the test organism.

2. The submitted efficacy data **supports** the use of the product, Brace, as a disinfectant with bactericidal activity on hard, non-porous surfaces in its ready to use form in the presence of a 5% organic soil load for a 2 minute contact time against the following:

   Escherichia coli (ATCC 11229) MRID 49599202

   According to the analysis of the active ingredient concentration for each product batch, the tested concentrations were at or below the lower certified limit of the active ingredient. Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the test organism.

3. The submitted efficacy data **does not support** the use of the product, Brace, as a disinfectant with bactericidal activity on hard, non-porous surfaces in its ready to use form in the presence of a 5% organic soil load with a 2 minute contact time against the following:

   Cronobacter sakazakii (ATCC 12868) MRID 49599203

   Testing of Batch 2028-029 conducted on 12/8/14 against *Cronobacter sakazakii* resulted in growth confirmed to be the test organism on 1/10 carriers. No protocol deviations and no contamination were reported, therefore repeat testing of this batch under the same test conditions was not warranted based on the failing result.

   However, the submitted efficacy data **supports** the use of the product, Brace, as a disinfectant with bactericidal activity on hard, non-porous surfaces in its ready to use form with no organic soil load and a 2 minute contact time against the following:

   Cronobacter sakazakii (ATCC 12868) MRID 49599203

   According to the analysis of the active ingredient concentration for each product batch, the tested concentrations were at or below the lower certified limit of the active ingredient. Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the test organism.

VII LABEL

1. The proposed label claims that the product, Brace, in its ready-to-use form is an effective one-step disinfectant with virucidal activity on hard, non-porous surfaces with a 1 minute contact time against the following:

   Enterovirus type 68 (ATCC VR-561)

   These claims are **acceptable** as they are supported by the submitted data.

2. The proposed label claims that the product, Brace, in its ready-to-use form is an effective one-step disinfectant with bactericidal activity on hard, non-porous surfaces with a 2 minute contact time against the following:

   Escherichia coli (ATCC 11229)

   These claims are **acceptable** as they are supported by the submitted data.

3. The proposed label claims that the product, Brace, in its ready-to-use form is an effective disinfectant with bactericidal activity on hard, non-porous surfaces with a 2 minute contact time against the following:

   Cronobacter sakazakii (ATCC 12868)

   This claim is **acceptable only for *pre-cleaned*** hard, non-porous, non-food contact surfaces. **Directions for the use of the product as a disinfectant against the above organism must always include instructions for pre-cleaning.** In the table on page 14 of the proposed label, this organism should include the directive **"<<Pre-cleaned surfaces>>"**, as for *Micrococcus luteus* (ATCC 14408).

4. In the footnote on each page of the proposed label, "x" should not exceed 99 when used as a placeholder for the number of organisms against which the product is effective (as on pages 10, 11, and 12). Only 99 unique organisms are listed in the table on pages 14-15. In addition, when "x" is used as a placeholder for the number of hard, non-porous surfaces or sites to which the product may be applied (as on page 10) only 90 such surfaces and 41 use sites are listed in the tables on page 16.

5. On page 5 of the proposed label, the statement "Lysol Protected Schools(!)" is unacceptable. "Protected" implies continued activity of the product for an indefinite period after its application, which is not supported by the submitted data.

6. On page 6 of the proposed label, the statement "Kills 99.9% of bacteria (in 30 seconds) and (eliminates) (neutralizes) (the toughest) (tough) odors on (soft surfaces) (fabrics) ({insert use site – see pg. 16})" should be qualified with the appropriate supporting claim qualifier from page 9 or with a term such as "odor-causing bacteria" to clarify the type of bacteria to which the claim refers.

7. On page 8 of the proposed label, the statement “Eliminates bacteria (in sports bags) (on gym bags)” should be qualified with the appropriate supporting claim qualifier from page 9 to clarify the type of bacteria to which the claim refers.

8. On page 12 of the proposed label, the statement “(Overall) Disinfection in 3 minutes(!)” should be qualified such that it applies only to organisms with disinfection claims with contact times less than 3 minutes as listed elsewhere on the label.

9. In the table on page 14 of the proposed label, “Corynebactium diptheriae” should be changed to “Corynebacterium diphtheriae”.

10. In the table on page 14 and on page 15 of the proposed label, “Klebsiella pneumonia” should be changed to “Klebsiella pneumoniae”.

11. In the table on page 15 of the proposed label, “Alternaria alternate” should be changed to “Alternaria alternata".